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Zoological Research

AN ALLOZYME ELECTROPHORESIS STUDY ON ELEVEN SPECIES OF MEGOPHRYINAE IN CHINA*

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Abstract Allozymes of eleven species of Megophryinae in China were examined electrophoretically to investigate genetic diversity and phylogenetic relationships. Fourteen enzymes, presumptively coded by 24 loci were detected to be variable. Gene frequencies of each population at each locus were presented. The commonly used measure of genetic diversity, the average heterozygosity (H) were calculated based on gene frequencies. The results indicated that Megophryinae had a high level of genetic diversity in amphibians, an average H of 0.18, ranging from 0.058 to 0.28. Nei's (1978) genetic distances(Nei's D) were calculated for all possible population pairs. A dendrogram of 13 populations representing 11 species, 3 genera of Megophryinae were derived and presented by using UPGMA, based on Nei's D. The assignment of *Ophryophryne* as a distinct genus were supported by an average Nei's D of 1.4067 which separated *O. microstoma* from all other populations.Subdivision of *Brachytarsophrys* from *Megophrys* was not supported by this study. Within *Megophrys*, three groups were recognized: ① *M. lateralis*, *M. giganticus* and *M. longipes*; ② *M. palpebralespineosa*, *M. boettgeri* and *M. parva*; ③ *M. minor* and *M. kuatunensis*. Three populations of *M. omeimontis* were closely related and share a clade independent from all other *Megophrys*, and *B. feae* as well.

Key words Amphibia, Pelobatidae, Megophryinae, Genetic diversity, Phylogenetic relationships, Allozyme electrophoresis, China

1 Introduction

Genetic variation is one of the fundamental parameters of evolutionary process. This is because the potential of a population is a function of the amount of genetic variation present in the population at a given time. The molecular approach has made possible an extensive comparison of organisms at levels that closely reflect gene products (Dessauer, 1974). Biochemical methods of both protein electrophoresis and DNA

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sequencing data has been used with increasing frequency to taxonomic and evolutionary problems in amphibians (see review of Nevo and Beiles, 1991). Starch gel electrophoresis (SGE) is a fairly common and simple technique that makes possible the study of protein variation with only a moderate investment. In this study, we present a allozyme electrophoresis approach to 11 species of Megophryinae in China.

The oriental megophryines are much diverse in external appearance, they are terrestrial or semifossorial and some have fleshly dorsolateral folds and / or supraeiliary processes. The Chinese megophryines were subdivided into two subfamilies by Dubois (1983, see Frost, 1985), Tian *et al* (1983). The subfamily Megophryinae governed four genera, i. e. *Megophrys*, *Brachytarsophrys*, *Atympanophrys* and *Ophryophryne*. Among them, the genus *Megophrys* is highly diversified, with over twenty species distributed in Southeastern Asia from India to China, south to Philippines, Sumatara, Java, Borneo and Natuna. Over half of its members occurred in China, some of them are endemic to China. The taxonomic and systematic problems of this group are far from being worked out. For example, the genus *Ophryophryne* (Boulenger, 1883) were considered synonym of *Megophrys* by some authors (Frost, 1985; Duellman *et al.*, 1986), but considered independent genus by others(Tian *et al.*, 1985; Zhao *et al.*, 1993). Further more, Liu *et al.*(1961) pointed out that morphological differences were existed among geographic populations of *M. omeimontis*, as well as in same case of *Brachytarsophrys carinensis* and *M. minor*. Hu (personal discussion, 1992), after comparing external and internal morphology of *B. feae* and *M. montana* (type species of *Megophrys*), suggested that they resembled each other.

The proposal of this work is not to solve all the above problems which may need considerable many works. This study is carried out to begin this process of understanding. Two questions are addressed: ① What is the phylogenetic relationships within this group? ② How much does the genetic diversity exist in the members of this group?

2 Materials and Methods

Between May, 1991 and August, 1993, a total of 188 specimens representing 13 populations of Megophryinae was collected during field trips; information on localities and sample sizes is given in table 1. Tissues of heart, liver and skeletal muscle were cut out immediately after sacrificing the animals and put in the liquid nitrogen(−196℃), and then brought to laboratory and maintained at −70℃ in refrigerator until use. Voucher specimens are deposited in Collection of Kunming Institute of Zoology, the Chinese Academy of Sciences.

Extracts were prepared by homogenizing each sample after method presented by Pasteur *et al.*(1988), and maintained at −10℃ until use. Fifteen specific enzymes were assayed by horizontal starch gel electrophoresis (SGE), and one, i. e. α−amylase, was assayed by polyacrylamide gel electrophoresis(PAGE). Enzymes, electrophoretic

conditions and staining references are listed in Table 2.

Table 1 Localities and sample sizes of thirteen populations of Megophryinae

Population and species	Locality	Elevation (approx. in m)	Sample size
1 *M. lateralis*	Tengchong, Yunnan	2340	20
2 *M. omeimontis*	Mt. Emei, Sichuan	750	20
3 *M. omeimontis*	Jingdong, Yunnan	2000	20
4 *M. omeimontis*	Pinbian, Yunnan	700–1100	3
5 *M. minor*	Jingdong, Yunnan	2000	20
6 *M. kuatunensis*	Dehua, Fujian	1300	12
	Chun-an,Fujian	1180	3
7 *M. palpebralespineosa*	Pinbian, Yunnan	700–1100	7
8 *M. boettgeri*	Chun-an, Yunnan	1180	15
9 *M. parva*	Mengla, Yunnan	600– 700	12
10 *M. longipes*	Hekou, Yunnan	120– 200	10
11 *M. gigantica*	Jingdong, Yunnan	2000	20
12 *O. microstoma*	Hekou, Yunnan	120– 200	4
13 *B. feae*	Jingdong, Yunnan	2000	22

Table 2 Enzymes, electrophoresis conditions and staining references

Enzyme	E. C. Number	Presumptive locus	Electrophoresis type and condition	Staining reference
Acid phosphatase	3.1.3.2	ACP-1	SGE,Tris-Citrate	P***
		ACP-2	pH7.0	
Alcohol dehydrogenase	1.1.1.1	ADH-1	SGE,Poulik*	P
		ADH-2		
Estrase	3.1.1.1	EST-1	SGE, Poulik	P
	3.1.1.2	EST-2		
Glucose dehydrogenase	1.1.1.47	GLC-1	SGE,Tris-Citrate	P
		GLC-2	pH7.0	
Glutamate dehydrogenase	1.4.1.2	GLD-1	SGE, Tris-Citrate	P
		GLO-2	pH7.0	
L-Lactate dehydrogenase	1.1.1.27	LDH-1	SGE,Poulik and	P
		LDH-2	Tris-Cirtrate,pH7.0	
Malate dehydrogenase	1.1.1.37	MDH-1	SGE, Tris-Citrate	P
		MDH-2	pH7.0	
Alkaline phosphatase	3.1.3.1	AKP-2	SGE, Tris-Citrate, pH7.0	P
α-Amylase	3.2.1.1	AMY-1	PAGE, Tris-Gly**	P
Octanol dehydrogenase	1.1.1.73	ODH-1	SGE, Poulik	P
		ODH-2		
Peroxidase	—	PER-1	SGE, Tris-Citrate,	S &P****
		PER-2	pH7.0	
Catalase	1.11.1.6	CAT-1	SGE, Poulik	S & P
Sorbitol dehydrogenase	1.1.1.14	SDH-1	SGE, Poulik	P
		SDH-2		
Superoxide dismutase*	1.15.1.1	SOD	SGE, Poulik	P

* Poulik = Tris-Citrate-Borate, pH8.7; ** discontinues, pH6.7 and 8.9;

*** Pasteur *et al.*, 1988; **** Shaw and Prasad, 1970.

Because malic enzyme(NADP-dependent malate dehydrogenase) and aldenyde oxidase were not considerably interpretable, these two enzymes were discounted from analysis. Thus, a maximum of 14 enzymes coded by 24 loci were used.

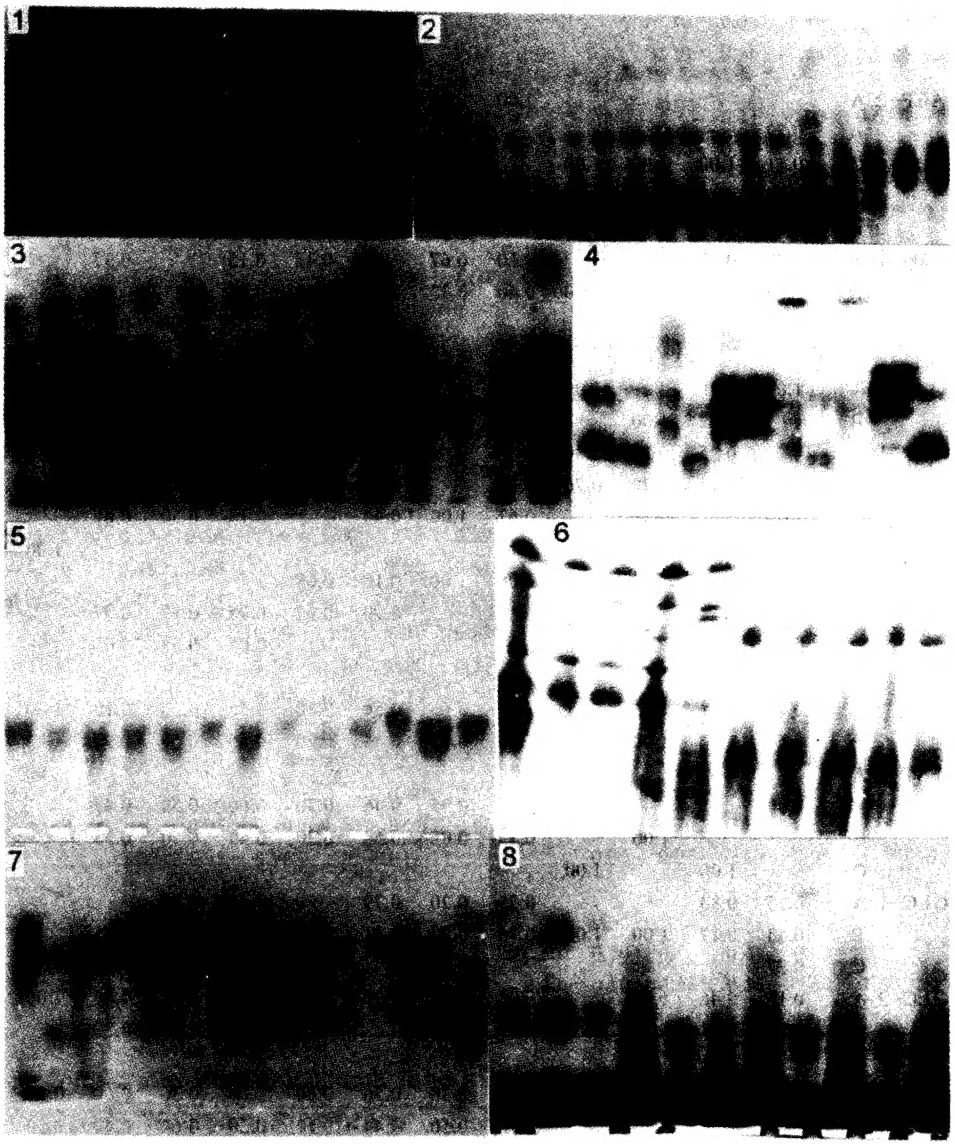

Figure 1 Some of the photographs of gels obtained by isozyme electrophoresis

1. AMY(α-Amylase); 2. LDH(L-Lactate dehydrogenase); 3. PER (Peroxidae); 4. EST(Esterase); 5. AKP(Alkaline phosphatase); 6. GLD(Glutamate dehydrogenase); 7. GLC(Glucose dehydrogenase); 8. ADH(Alcohole dehydrogenase)

Table 3 Allelic frequencies for variabe loci

Locus		Populations												
		1	2	3	4	5	6	7	8	9	10	11	12	13
ACP-1	A		0.50	0.44	0.33		1.00							
	B	0.85	0.50	0.56	0.67	1.00			0.95			1.00		
	C	0.15						1.00	0.05	0.10	0.83			1.00
	D									0.90	0.17			
	E												1.00	
ACP-2	A					1.00	1.00		0.23					
	B	0.72							0.65	0.90	1.00	1.00		
	C	0.11	1.00	1.00	1.00			1.00	0.12	0.10				
	D	0.17												1.00
	E												1.00	
ADH-1	A	1.00	0.42			0.60	0.67		0.47	0.15		0.37		0.56
	B		0.58	1.00	1.00	0.40	0.33	1.00	0.53	0.85				0.44
	C										1.00	0.63		
	D												1.00	
ADH-2	A	1.00	0.38		1.00			1.00	0.44	0.05	0.93	0.30	1.00	
	B		0.62	1.00		1.00	1.00		0.56	0.95	0.07	0.70		1.00
AKP-1	A		0.25	0.30	0.30				0.33	0.23		0.05		
	B		0.75	0.70	0.70	0.38		0.56	0.67	0.64	0.50	0.10	1.00	
	C	1.00				0.62	1.00	0.44		0.13	0.50	0.85		1.00
AMY-1	A												1.00	
	B		1.00	1.00	1.00	1.00	1.00	0.10	0.08					
	C	0.87						0.90	0.92	0.95	0.17	0.37		
	D	0.13								0.05	0.83	0.63		
	E													1.00
EST-1	A	0.46					0.65	0.56	0.80	0.45	0.50	0.10	1.00	0.21
	B	0.54	1.00	1.00	1.00	1.00	0.35	0.44	0.20	0.55	0.50	0.90		0.79
EST-2	A												1.00	
	B	1.00				0.67	0.95	0.46	0.70	0.60	0.50	0.47		1.00
	C			1.00		0.33	0.05	0.54	0.30	0.40	0.50	0.53		
	D		1.00		1.00									
GLC-1	A	0.15	0.83			0.25	0.20	0.79		0.54	0.83			
	B	0.58	0.17	1.00	1.00	0.75	0.70	0.21	0.85	0.46	0.17	0.71		0.50
	C	0.27					0.10		0.15			0.29	1.00	0.50
GLC-2	A	0.13	0.46	1.00		0.08	1.00	1.00	1.00			0.80	1.00	
	B	0.87	0.54		1.00	0.92				1.00	1.00	0.20		0.50
	C													0.50
GLD-1	A	0.36	0.35	0.05	0.83	0.17	0.38	0.56	0.60		0.36	0.60	0.50	0.19
	B	0.64	0.54	0.10	0.17	0.78	0.50	0.44	0.17	0.20	0.64	0.40	0.50	0.81
	C		0.11	0.85		0.09	0.12		0.23	0.80				
GLD-2	A	1.00	1.00	1.00	1.00								1.00	0.50
	B					1.00	1.00	1.00	1.00	1.00	1.00	0.67		0.50
	C											0.33		
LDH-1	A	0.05												1.00
	B	0.95				1.00	0.08	1.00	1.00	1.00	0.19	1.00		
	C		1.00	1.00	1.00		0.92				0.81			
	D												1.00	

(continued)

Locus		Populations												
		1	2	3	4	5	6	7	8	9	10	11	12	13
LDH-2	A					0.10							1.00	
	B	1.00	0.69	1.00	1.00	0.90	0.22	1.00	1.00	1.00	1.00	1.00		1.00
	C		0.31				0.78							
MDH-1	A	0.40			1.00	0.10				0.30	0.44	0.18	0.67	
	B	0.60	0.31	1.00		0.60	1.00	1.00	0.82	0.70	0.56	0.61	0.33	0.29
	C		0.69			0.30			0.18			0.21		0.71
MDH-2	A		0.62	0.25		0.60	0.70		1.00		0.11	0.07	0.67	
	B	1.00	0.27	0.12	0.50	0.40	0.30	1.00		1.00	0.89	0.72	0.33	1.00
	C		0.11	0.63	0.50							0.21		
PER-1	A	1.00	1.00	1.00	1.00			1.00	0.10	1.00		0.05	1.00	0.33
	B								0.90		1.00	0.95		0.67
	C					1.00	1.00							
PER-2	A									1.00				
	B	0.73							0.25		1.00	0.78	1.00	0.75
	C	0.27	1.00	1.00	1.00			0.85	0.43			0.11		0.25
	D					1.00	1.00	0.15	0.32			0.11		
SDH-1	A	0.45		1.00	1.00		0.25	0.37	0.53	0.90	0.79	0.68		
	B	0.55	1.00			1.00	0.75	0.63	0.47	0.10	0.21	0.32		1.00
	C												1.00	
SDH-2	A	0.55	0.54	1.00	1.00		0.39	0.31	0.23	0.05		0.50		0.10
	B	0.45	0.46			1.00	0.61	0.69	0.77	0.95	1.00	0.50	1.00	0.90
ODH-1	A	0.13	0.63						0.38					
	B	0.87		0.50		0.10	0.83	1.00	0.62		1.00	1.00	1.00	1.00
	C		0.37	0.50	1.00	0.90	0.17			1.00				
ODH-2	A		0.33	0.50	0.50	0.17	0.39		0.15			1.00		1.00
	B	1.00	0.67	0.50	0.50	0.83	0.61	1.00	0.85	1.00	1.00			
	C												1.00	
SOD	A					0.80	0.95							
	B		0.05	0.10	1.00	0.20	0.05	1.00	0.95	0.75	0.75		1.00	
	C	1.00	0.95	0.90					0.05	0.25	0.25	0.95		0.75
	D											0.05		0.25
CAT	A	0.85										0.40		0.50
	B	0.15						0.50	0.50	0.50	0.44	0.60		
	C		1.00	1.00	1.00	0.60		0.50	0.50	0.50	0.56		1.00	0.38
	D					0.40	1.00							0.12

Allele frequencies were calculated, and a chi-square test were used to test for deviation from Hardy-Weinburg equilibrium. The Nei's genetic distance (D) was calculated for each possible population pair. Because sample sizes were relatively small (most were between 10 and 20), the Nei's D were calculated by using the modified method for small size presented by Nei(1978).

3 Results

In most cases, the pattern of bands observed electrophoretically at any given locus was similar to that seen in other vertebrates(for some of the gels, see Fig. 1). The gels

were interpreted according to Pasteur *et al.* (1988) and Harris *et al.* (1976). Gene frequencies of each population were calculated and listed in the Table 3. Observed heterozygosity of each population at each locus was tested, and in no case was the value of heterozygosity observed per-locus significantly different from that expected under Hardy-Weinburg law. The average heterozygosity (H) of each population were calculated, and the result indicated that the level of H of Megophryinae was very high, with a mean value of 0.18, ranging from 0.058 to 0.28(see Tab. 4).

Table 4 Average heterozygosity (H) of 13 populations representing 11 species of Megophryinae

population	1	2	3	4	5	6	7	8	9	10	11	12	13
H	0.213	0.253	0.120	0.089	0.117	0.194	0.172	0.239	0.195	0.214	0.285	0.058	0.209

Nei's (1978) genetic distance (D) was calculated for each possible population pair, and given in Table 5. A dendrogram was derived from this D matrix by using UPGMA(Fig. 2). We can see that *Ophryophryne microstoma* share one clade, separate from other populations with an average genetic distance of 1.4067; the remaining populations share the other clade, within this clade, the average genetic distance between any population pair is below 1.0. Therefore, this result supports the subdivision of *Ophryophryne* from *Megophrys* as a distinct genus. But it is not the same case for *Brchytarsophrys*.

Table 5 Nei's genetic distance of 13 populations representing 11 species of Megophryinae

	1	2	3	4	5	6	7	8	9	10	11	12	13
1	0	0.8300	0.8163	0.8460	0.7413	0.9272	0.4998	0.5480	0.6534	0.5253	0.3145	0.1330	0.4892
2		0	0.3046	0.3573	0.6689	0.8842	0.6662	0.7944	1.1101	0.9857	0.9091	1.3037	0.8038
3			0	0.3125	0.8127	0.8226	0.6014	0.6811	0.8003	1.0861	0.9297	1.3602	1.0708
4				0	0.6913	0.9136	0.7844	0.8860	0.8000	1.0190	1.1123	1.2825	1.2595
5					0	0.3384	0.7080	0.4677	0.5299	0.7711	0.6369	1.7702	0.7502
6						0	0.7844	0.6034	0.9983	0.9306	0.7494	1.5729	0.7573
7							0	0.3320	0.4386	0.5869	0.6482	0.9906	0.7424
8								0	0.4565	0.5125	0.3772	1.1571	0.8097
9									0	0.5149	0.6892	1.4729	0.9128
10										0	0.4379	1.3834	0.6403
11											0	1.4447	0.5269
12												0	1.3747
13													0

4 Discussion

4.1 Heterozygosity

The average heterozygosity(H) is commonly used as a measure for genetic diversity. The levels of genetic diversity measured by H in amphibians are varied non-randomly on a massive scale among taxa (families, genera, species), as well as among biotic factors(ecological, demographic, and life history). H is structured both taxonomically and biotically. H is also higher in amphibians as compared with other vertebra(Nevo *et*

al., 1991). The majority of amphibians hither-to studied had a level of H below 0.13, but actually a few taxa had a level of exceeding 0.20 (Green *et al.*, 1988). In the present study, H of Megophryinae is very high. This is interesting but difficult to understand, because references of the ecology of Megophryinae is rare. However, Liu *et al*(1961) pointed out that external morphological divergences were exited in some species of *Megophrys*. And a recent study revealed that skeletal divergences were also existed in inter- and intra-populations of *M. lateralis*. So, genetic diversity may be congruent with morphological divergence in *Megophrys*.

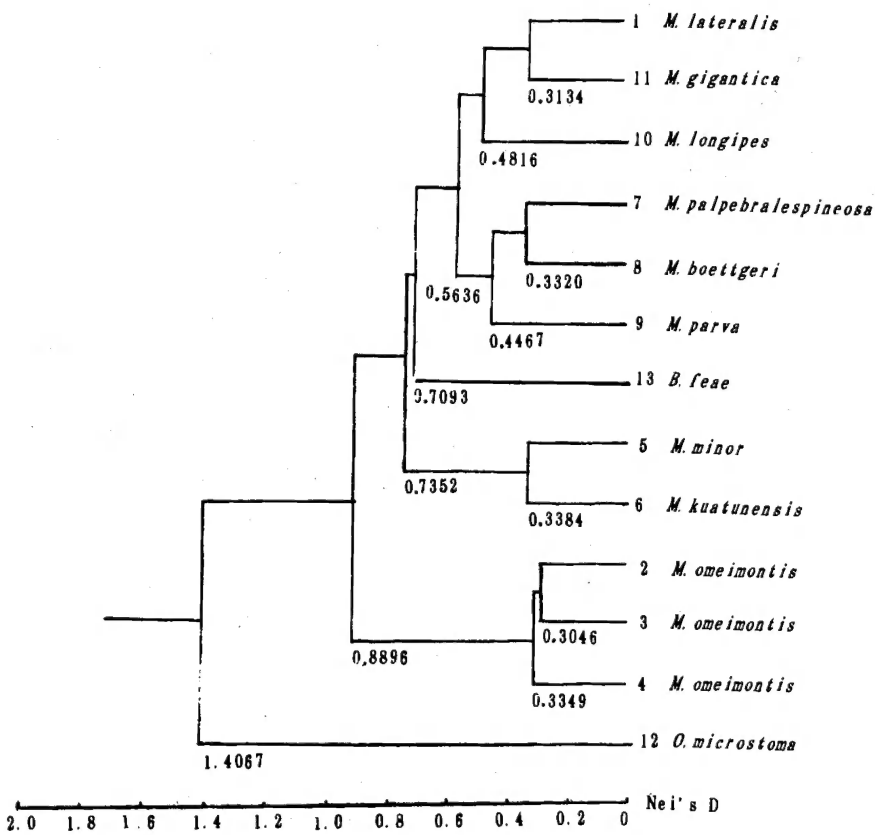

Figure 2　Dendrogram of 13 populations of 11 species of Megophryinae in China, based on Nei's genetic distance, derived by UPGMA

4.2 Biochemical systematics

The family Pelobatidae is formerly divided into two subfamilies, Pelobatinae and Megophryinae. Recently, the latter was further arranged into two subfamilies, i.e. Megophryinae and Leptobrachiinae(Dubois, 1983, see review of Duellmam, 1986), the present study concentrated on Chinese members of the former. The systematics of this group are confusing, and the phylogenetic relationships within it is far from being

worked out. *O. phryophryne* was regarded as a distinct genus by Liu *et al*(1961), Tian *et al*(1985), Fei *et al*(1990) and Zhao *et al*(1993), but regarded as subgenus following Dubois (1980). Duellman and Trueb(1986) and Frost(1985) did not subdivided *Ophryophryne* from *Megophrys*. *Brachytarsophrys carinensis* was also divided from *Megophrys* based on external and internal morphological differences from other members of *Megophrys*(Tian *et al.*, 1986). But recently, Hu Qixiong(1992, personal discussion), after comparing morphology of *B. feae* with *M. montana*, suggested that these two species resembled each other, and suspected the validity of *Brachytarsophrys*.

Thorpe(1983), using Nei's genetic identity, I, found that members of the same vertebrate species almost always have identities > 0.900 (equivalent to Nei' s D < 0.105), while members of different species within genera characteristically have identities > 0.350 (equivalent to Nei's D < 1.050). (Green *et al*, 1988). Therefore, the average Nei's D of 1.4067 which separate *O. microstoma* from the remaining species supported the subdivision of *Ophryophryne* from *Megophrys* as a distinct genus. This result was also convinced by tadpole oral characteristics presented by Huang *et al*(1991). As for the *Brachytarsophrys*, it is not the same case, an average Nei' s D of below 0.8896 which separate *feae* from other *Megophrys* did not support the subdivision of *feae* from *Megophrys*.

In *Megophrys*, three groups with intra-group close relationships are recognized: ① *M. lateralis*, *M. giganticus* and *M. longipes*, where the former two are closely related, with an average Nei' s D of 0.3145, while the latter was separated from the former two by an average Nei' s D of 0.4816; ② *M. palpebralespineosa*, *M. boettgeri* and *M. parva*, where the former two are each other' s close relatives, with a Nei' s D of 0.3320, an average Nei' s D of 0.4467 separate the latter one from the former two; ③ *M. minor* and *M. kuatunensis* are closely related, with a Nei' s D of 0.3384. The above groupings are, at least to some extent, congruent with morphological data (Yang *et al.*, in preparation).

It is special for the case of *M. omeimontis*. Three geographical populations of this species examined here share a clade, this clade separated it from all species of *Megophrys*, and *B. feae* as well, with a considerable high average Nei' s D, 0.8896. Among the three populations of this species, samples from Jingdong, Yunnan and Mt. Emei, Sichuan (type locality) are more closely related each other than to samples from Pinbian, Yunnan. Because *M. omeimontis* is widely distributed in the southwestern China, divergences between populations are well known(Liu *et al.*, 1961). Samples from Jingdong, Yunnan are regarded as subspecies by Fei *et al*(1990). A further study concentrating on various geographic populations of this species is necessary for the future to investigate population divergences and phylogeny.

References

Ayala F J, 1983. Genetic polymorphism: from electrophoresis to DNA sequences. *Experientia*, **39**: 813–823.

Dessauer H C, 1974. Biochemical and immunological evidences of relationships in Amphibia and Reptilia. In: Wright, C. A.(ed.): Biochemical and immunological taxonomy of animals. London New York: Academic Press. 177–242.

Dubois A, 1980. Classification generique et subgenerique des Pelobatidae Megophryinae. *Bull. Soc. Linn.* Lyon, **49**(8): 649–656.

Duellman W E, Trueb L, 1986. Biology of amphibians. McGraw–Hill. New York. 1–670.

Fei L, Ye C Y, Huang Y Z, 1990. Key to Chinese Amphibia. Chongqing: Chongqing Branch, Sci. Technol. Literature Press. 1–364.

Frost D. (ed.), 1985. Amphibian species of the world, Lawrence, Kansas: Assoc. Syst. Coll.

Green D M, Nussbaum R A, Yang D T, 1988. Genetic diversity and heterozygosity among frogs of the family Sooglossidae. *Herpetologica*, **44**(1): 113–119.

Harris H, Hopkinson D A, 1976. Hand–book of enzyme electrophoresis in human genetics. Amsterdam. North–Holland Publ. Co.

Huang Y Z, Fei L, Ye C Y, 1991. Studies on internal oral structures of tadpoles of Chinese Pelobatidae. *Acta Biologica Plateau Sinica.*, (10): 71–99. (in Chinese with English abstract).

Liu C C, Hu S Q, 1961. Tail–less amphibians of China, Beijing: Science Press.(in Chinese).

Nei M, 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**: 583–590.

Nevo E, Beiles A, 1991. Genetic diversity and ecological heterogeneity in amphibian evolution. *Copeia*, (3): 563–592.

Pasteur N, Pasteur G, Bonhmme F *et al*, 1986. Practical isozyme genetics. West Sueex, England: Ellis Horwood Lt. 1–215.

Shaw C R, Prasad R, 1970. Starch gel electrophoresis of enzymes: A compilation of recipes. *Biochemical Genetics*, **4**: 297–320.

Tian W S, Hu Q X, 1983. Taxonomic study on genus *Megophrys*, with description of two new genera. *Acta Herpetol. Sinica*, Chengdu, **2**(2): 41–48. (in Chinese with English abstract)

Tian W S, Jiang Y M.(eds.), 1986. Identification manual for Chinese amphibians and reptiles. Beijing: Science Press. 1–164 (in Chinese).

Zhao E M, Adler K, 1993. Herpetology of China. Published by Society for the study of amphibians and Reptiles in cooperation with Chinese Society for the study of Amphibians and reptiles. Oxford, Ohio, U. S. A., 1–522.

中国角蟾亚科十一种的同工酶电泳研究

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摘要 本文用同工酶电泳的方法分析了中国产角蟾亚科 3 属 11 种，共 13 个地理种群样本，以探讨它们的遗传多样性及系统关系。用于分析的酶共 14 种，由 24 个基因位点编码。经过酶带的分析和计算，得出了每个种群样本在每个基因位点上的等位基因频率(见表 3)。根据基因频率的分布，计算得出了各种群样本的基因多样性指数——平均杂合率(H)(见表 4)，结果表明，角蟾亚科的 H 值平均值为 0.18，在两栖动物中是属于很高的遗传多样性水平，根据基因频率的分布，计算了所有种群样本间的 Nei's 遗传距(见表 5)，并通过类平均法(UPGMA)重建了进化树(dendrogram)。分析结果支持将拟角蟾属(*Ophryophryne*)从角蟾属(*Megophrys*)中分出为独立属的观点；但是对短腿蟾属(*Brachytarsophrys*)是否为独立属提出了疑问。在角蟾属(*Megophrys*)中，可以明显地看出 3 个组，它们中的种间亲缘关系比较近，这 3 个组分别是：① 白颌大角蟾(*M. lateralis*)、大花角蟾(*M. giganticus*)和长肢角蟾(*M. longipes*)；② 粗皮角蟾(*M. palpebralespinneosa*)、淡肩角蟾(*M. boettgeri*)和凹顶角蟾(*M. parva*)；③ 小角蟾(*M. minor*)和褂墩角蟾(*M. kuatunensis*)。峨嵋角蟾(*M. omeimontis*)3 个地理种群聚为一支，互相间有一定的遗传差异，这与该种地理种群间的形态差异相一致。我们认为，该种不同地理种群间的比较还有必要进一步深入研究。

关键词 锄足蟾科，角蟾亚科，遗传多样性，系统进化，同工酶电泳